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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 12 NOVEMBER 1954 No. 11

TRENDS IN NUTRITIONAL RESEARCH

That research in any field of science is likely to become channeled so as to contribute its benefits from the velocity rather than by the breadth of the stream is an observation which must have been made many times. These swift moving trends are of interest from several points of view: (1) What starts one: a brilliant discovery, a strong and persuasive personality, a sudden improvement in method? (2) What keeps it going: a logical progression from one vantage point to another, the band wagon complex, the strategic importance of the subject? (3) How long is it likely to continue? Is there a time curve of characteristic dimensions? (4) Can scientists be sampled as to behavior, as movie-goers can be, so as to lend themselves and their productivity to prediction? (5) Finally, what is its contribution?

The present interest in isotope research in many biologic sciences traces directly to the explosions heard round the world in August 1945, for the laboratory methods devised in that great strategic undertaking resulted in making nearly all the chemical elements in their radioactive forms available at reasonable prices, for an infinity of inquiries, within less than two years from its culmination. The atomic bomb clearly started a trend in nutritional research. What will be its outcome?

For examples of some trends, about which there is no doubt of the outcome, for their histories are made, consider two on the nutritional front which were already evident when the writer enlisted for the duration in 1903.

I

Dr. Chittenden had in 1875 brought with him from Kühne's laboratory at Heidelberg a consuming interest in digestion and metabolism, particularly of the proteins, and had cooperated with his old professor on several papers after becoming established at Yale. The first paper accepted for publication in volume I of the *American Journal of Physiology*, which appeared 57 years ago next January, was one on protein metabolism by R. H. Chittenden and W. J. Gies. Papers on proteins were still pouring from his laboratory in 1903, and in 1904 his interest culminated in his book on "Physiological Economy in Nutrition" and in 1907 the book entitled "Nutrition of Man."

In 1891 L. B. Mendel graduated at Yale with a Bachelor's degree and immediately began the study of physiological chemistry under Professor Chittenden. He soon became an assistant in the protein investigations, then a colleague, and in 1912 succeeded to the professorship. These two, Chittenden and Mendel, made the Sheffield School laboratory known all over the world wherever physiology was being read. There were many other students of the two leaders, who in their turn have become leaders, and the subjects of their dissertations were by no means confined to proteins and their metabolism, but these were dominant themes.

At Baltimore in 1938 when Professor Chittenden attended the first advanced Federation day meeting of the Institute of Nutrition, and found himself in a large assembly hall well filled, he asked: "Who are all these people?" The answer was: "Everybody in the Federation interested in Nutrition. You see what you started." He only smiled and shook his head. That same evening when he was being introduced to some 300 people in attendance at the dinner in his honor, everybody present who had been a student of Chittenden, or a student of a student and finally a student of a student of a student of

his, was asked to stand; the number comprised well over half the total. This illustrates better than any other brief way how extensive had become the interest in this country descended directly from Chittenden's own interest. A personality with a persuasion, plus continuing profitable progress, maintained this stream running full and strong.

In 1905—just between Chittenden's two influential books—came the publication of Folin's analytic methods for measurement of the nitrogenous and sulfurous constituents of the human urine and his challenging ideas regarding protein metabolism. Laboratories of physiology and biochemistry (as they were beginning to be called) all over the country took up the analysis of the urines of mammals and men under a great variety of dietary, physiologic and pathologic conditions to learn how the partitions of the nitrogen- and sulfur-containing substances were affected by such conditions. One needs only glance through the first ten volumes of the *Journal of Biological Chemistry* and the second ten volumes of the *American Journal of Physiology* to observe how widespread was the interest in the details of protein metabolism. The creatine and creatinine fractions alone, both methods and results, comprise the subject matter of at least two dozen papers in a period of about five years. The deluge was unabated in the next five. New methods and new ideas started the trend and new developments maintained it.

Starting with the first volume of the *Journal of Biological Chemistry* (1905–6) came the series of papers by H. D. Dakin and associates at the Herter laboratory on the oxidation of amino acids, and with the third volume (1907) the series by A. E. Taylor and by T. B. Robertson on the synthetic action of the proteolytic enzymes. About the same time T. B. Osborne began publication on the hydrolysis of a long series of vegetable proteins and identification of the resulting amino acids. In the sixth volume of the *Journal of Biological Chemistry* there are ten papers on amino acids by Dakin, Wakeman, P. A. Levene, D. D. Van Slyke and A. P. Mathews, seven papers on enzymes, three on the characterization of proteins, and three on purines. The next three or four volumes maintained the pace. All these point up the intense preoccupation of these years with proteins, their constituents and what happens to them after absorption. The stimulus came in part from European savants—notably Emil Fischer and Kossel—concerning the nature of the constitution of proteins, but in part it was the successful invention of new methods and the equipment of new laboratories here at home which implemented the ambitious endeavors of American physiologists and biochemists to learn what happens to food after it is eaten.

II

The year Mendel began work with Chittenden, Graham Lusk returned from Munich and obtained an appointment to teach physiology in the Yale Medical School "at the princely salary," as he was fond of saying, "of $500." He and Mendel became fast friends and as Lusk's mother was a Chittenden, it was a comfortable place to start a career. Lusk was imbued with the teachings of his "master and friend," Carl Voit, regarding metabolism of the principal foodstuffs, and with the ideas of his friend and fellow student, Max Rubner, regarding energy metabolism. The first and subsequent editions of his book, "The Science of Nutrition" (1906 to 1924), expounded these principles of both his teacher and his friend.

Lusk had brought with him from Europe a small model of Voit's respiration apparatus, which he set up at University and Bellevue Hospital Medical School, where Lusk became professor of physiology in 1898. This apparatus saw much service in the elucidation of the energy metabolism of diabetes, and of pregnancy in the dog.

W. O. Atwater also had studied with Voit

at Munich and became familiar with Rubner's work at Marburg on energy metabolism. With the help of Rosa, the physicist at Wesleyan University, Atwater built at Middletown (1897) the first direct-indirect calorimeter for man, fashioned in many respects after the Rubner dog calorimeter. It was remodeled with the help of F. G. Benedict to permit measurement of oxygen absorption (1905), and when Benedict moved to Boston to occupy the Carnegie laboratory, several different calorimeters were constructed on the same lines to permit measurement of heat production in man, while lying, sitting or working in an upright position. In the next two decades a large number of masterly monographs, published by the Carnegie Institution of Washington, flowed from this laboratory, covering many phases of energy metabolism and heat regulation in a large number of men, women, and children.

When Lusk went to Cornell University Medical College in 1909 he began at once, with the help of H. B. Williams, the construction of a small direct-indirect calorimeter after Benedict's model, suitable for dogs or small children; and in 1912 with the help of G. D. Soderstrom he and E. F. DuBois set up in Bellevue Hospital a bed calorimeter designed to study heat production and all phases of energy metabolism in bedridden patients. From these two laboratories between the years 1912 and 1930 appeared a nearly equal number of papers on animal calorimetry and clinical calorimetry, to the total of almost one hundred. The same programs were continued in the Department of Medicine by DuBois and J. D. Hardy at the New York Hospital and Cornell Medical Center until recently.

Armsby's direct-indirect calorimeter for large farm animals set up at Pennsylvania State College (1903) completed the armaments for an attack on the many phases of energy metabolism all along the mammalian front. After Armsby's death the work went forward under his successors, E. B. Forbes and R. W. Swift, the emphasis being shifted to the indirect measurement of heat production in the rat. Samuel Brody at the University of Missouri also made many adaptations of the bag method of collecting respiratory products of farm animals for analysis and evaluation. His authoritative book on "Bioenergetics and Growth" contains a multitude of results from his numerous observations.

The contributions from all of these laboratories, supported by funds from the U. S. government, from the Carnegie Institution of Washington, and from the Russell Sage Institute of Pathology of New York, as well as the universities where they were located, established and maintained a trend in nutritional research from the beginning of the century to recent times which widened and enriched enormously our knowledge of the processes and principles of vital energetics. Although the number of persons engaged in these investigations was not large, the broad conceptions contributed by the leaders supplemented by skilled technical assistance and freshened by the eagerness of younger minds from time to time, kept the interest at fever pitch among the students in this field.

It was not to be expected that these same methods and concepts would suffice to maintain such interest indefinitely. Already many calorimeters are standing idle. But the respiratory metabolism of tissue slices and of small organisms, carrying the quest as nearly as possible to individual cells, is applying many of the principles and concepts learned from the vital energetics of mammals and men. Certainly the determination of respiratory quotients in a germinating seed, a developing embryo, or a single cell portrays processes of oxidation akin to those demonstrated for a man.

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BODY WATER AND BODY COMPOSITION

Determination of the principal fluid spaces of the body is useful in a variety of problems of human biology. D. L. Hutchinson, A. A. Plentl, and H. C. Taylor (*J. Clin. Invest.* **33,** *235 (1954)*) examined changes in total body water, determined as deuterium oxide space, and total body solids (total weight minus water) in a series of uncomplicated pregnancies. In many clinical conditions, medical and surgical, body water measurements help in understanding weight changes which may occur without any readily noticeable signs of edema or dehydration. In addition, the data on body fluids may be used for the estimation of body fat, the most variable component of the human body. Information on fat-free body mass may be helpful in the interpretation of variations in basal oxygen consumption and provide a better criterion for the dosage of certain drugs.

Methods for the study of body water compartments (space technics), based on a dilution principle, were summarized by N. Deane (*Methods in Medical Research* **5,** *155 (1952)*). The volumes determined by the dilution of inulin and sucrose are considered as approximating more nearly the "true" extracellular fluid volume in man than the volumes of distribution of other test substances, such as sodium thiocyanate. Reference was made also to the determination of total body water by the isotope technics (dilution of deuterium oxide and tritium). The intracellular water is a derived value (total minus extracellular) and its precision depends on the accuracy of the primary methods. This should be kept in mind in evaluating alterations in cellular hydration (intracellular edema or dehydration).

G. J. Hamwi and S. Urbach (*Metabolism* **2,** *391 (1953)*) presented a method for the estimation of body fat and some of the other major compartments of the body, based on the determination of total body water (from antipyrine dilution) and extracellular water (from thiocyanate dilution). In principle, the procedure is similar to that outlined by R. A. McCance and E. M. Widdowson (*Proc. Roy. Soc. (London) B* **138,** *115 (1951)*). Theoretically, the total body mass is broken down into fat, cell mass (cell solids and intracellular water), extracellular water, and bone minerals (estimated as 7 per cent of the fat-free body mass). In practice, fat is calculated as the difference between total body weight and fat-free body weight (estimated from total body water, on the assumption that water makes up 73.2 per cent of the fat-free body mass). The biologic "constant" was taken from N. Pace and E. N. Rathbun (*J. Biol. Chem.* **158,** *685 (1945)*) who obtained it as the average value of original measurements and data from the literature on the water content of fat-free mass in six different kinds of animals. In subsequent work the value 71.8 per cent was used (E. F. Osserman, G. C. Pitts, W. C. Welham, and A. R. Behnke, *J. Applied Physiol.* **2,** *633 (1950)*).

Hamwi and Urbach (*loc. cit.*) presented systematic data on a small group of normal subjects and compared them with variations found in abnormal nutritional and pathologic states. The average fat content in the "normal" group (consisting of 2 males and 3 females with average age of 43 years) was given as 27 per cent, in patients emaciated owing to chronic undernutrition as 6.1 per cent, in obese subjects as 39 per cent of body weight. In line with the findings of other investigators, the extracellular fluid in emaciated patients was higher than in the normal subjects (31.4 versus 21.7 per cent). The relative cell mass was estimated as 46 per cent in the normal subjects, 56 per cent in the chronically undernourished and 37 per cent of body weight in the obese.

Of some interest also are data on aging, even though the group studied is very small and heterogeneous, containing both men and women. The elderly patients tend to have a

higher fat content (31.7 per cent of body weight on the average). The extracellular water compartment was larger (26.5 per cent) than in younger adults. Intracellular water was low (23 per cent as compared with 32 per cent in the younger group). The non-fat cell solids in these two groups were estimated as 13 and 16 per cent, respectively.

Data are presented on 2 patients, indicating an increase in intracellular water and the estimated cell mass resulting from the administration of testosterone. The authors feel that this strengthens the validity of the hydrometric technic as a method for the study of metabolic phenomena and changes in protoplasmic mass. However, the experimental errors of the technic are fairly large. In order to be able to examine satisfactorily not only the gross changes in body composition resulting from maintenance on drastically altered dietary intakes but also the alteration in body composition in response to minor variations in dietary regimen, to disease, injury and therapy, there is a need for development of technics of greater accuracy. The magnitude of errors resulting from the variability of the biologic "constants," such as the water content of the fat-free body mass, was not estimated by Hamwi and Urbach (*loc. cit.*).

G. L. Pawan (*Arch. Middlesex Hosp.* (*London*) **4,** *119 (1954)*) reported additional experiences with the routine use of urea, taken by mouth, for measuring total body water. Normally, urea is absorbed from the gut and distributed throughout the body water between one and two hours after ingestion. If abnormal water depots are present, more than three hours may be needed for an even distribution of urea throughout the body fluids. Measurements made on normal subjects at weekly intervals indicate good reproducibility with the careful use of the urea method, including abstinence from a protein meal from the evening preceding the test. The absolute values were somewhat lower than those obtained by a derivative of antipyrine, N-acetyl 4-aminoantipyrene (NAAP). In normal subjects the total body water represented 62 per cent of the body weight. With increasing adiposity this percentage decreased. In a group of 8 obese individuals the average value was 45 per cent, decreasing in grossly obese subjects (244 and 272 kg.) to 30 and 28 per cent. The urea dilution method, used in England by McCance and Widdowson (*loc. cit.*), is convenient and appears to be accurate. However, additional data are needed to examine both reproducibility of the values and the precision measured against determinations of total body water with isotopic modifications of water.

THE ROLE OF DIET IN THE MANAGEMENT OF ATHEROSCLEROSIS

Recent clinical and experimental studies in the field of atherosclerosis have excited interest in the possibility that the elevated serum lipids sometimes associated with this disease in man may be lowered by dietary means (J. W. Gofman, *Bull. N. Y. Acad. Med.* **28,** *279 (1952)*; A. Keys, *see Nutrition Reviews* **12,** *270 (1954)*). If control of serum lipids through dietary management, in turn, could be shown to influence favorably the course of such serious manifestations of atherosclerosis as coronary heart disease, it would be of great importance to practitioners of medicine and public health. Continuing scientific inquiry into this subject is thus of prime importance, particularly since our present knowledge suggests that more than one dietary constituent affects lipid transport and hence may modify the course of atherosclerosis.

J. W. Gofman, A. Tamplin, and B. Strisower (*J. Am. Dietet. Assn.* **30,** *317 (1954)*) have recently reviewed their own data bearing on the relationship of certain

species of serum lipoproteins to clinical coronary heart disease and the effect of fat and caloric restriction upon these lipoproteins and the course of the illness. In earlier communications, this group (Gofman, *Bull. N. Y. Acad. Med., loc. cit.*) emphasized the importance of the band of lipoproteins extending from S_f 20 to 100, which accounts for only 15 per cent of the total serum cholesterol, yet seems to correlate best with clinical evidences of atherosclerosis. These workers are now employing a formula which weights certain species of lipoproteins in the whole range between S_f 0 to 400 to obtain what they term the *Atherogenic Index* (A. I.). This index is said to correlate with "coronariness" or the tendency to develop coronary heart disease better than any single band of lipoproteins. Frequency distribution plots of A. I. values for male subjects aged 40 to 59 who are clinically well, and for patients with coronary heart disease in the same age group show a considerable overlap but present statistically different means, the coronary group being displaced toward higher A. I. values. From these curves and from the known rate of incidence of coronary heart disease in the United States per year, Gofman *et al.* calculate the probability that a clinically well individual 40 to 59 years of age with a given A. I. will develop coronary heart disease within one year. The probabilities of developing such illness ranged from 0.8 to 4 per cent, according to the A. I. value chosen. Although it is recognized that coronary heart disease may be clinically unapparent for long periods and may account for a portion of the clinically well individuals with elevated A. I. values, it is more difficult to explain why some patients with coronary heart disease have very low A. I. values unless other factors are of critical importance in the pathogenesis of this disease.

Obesity, which is one form of overnutrition, is associated on the average with an increased mortality from coronary heart disease (D. B. Armstrong, L. I. Dublin, G. M. Wheatley, and H. H. Marks, *J. Am. Med. Assn.* **147,** *1007 (1951)*; *Nutrition Reviews* **10,** *138 (1952)*) and with postmortem evidence of increased atherosclerosis (S. L. Wilens, *Arch. Int. Med.* **79,** *129 (1947)*). Gofman and his group have shown that the mean atherogenic indices in groups of clinically well subjects rise with increasing degrees of overweight and provide the bases for calculations of the probability of the overweight groups developing coronary heart disease which are in reasonably good agreement with actuarial data from insurance company records.

Gofman *et al.* point out, however, that the correlation between degree of overweight and A. I. in individual cases is not so high as when average data are taken. This suggests that overweight per se is not the only significant factor in the causation of coronary artery disease in overweight patients. It is probable that the dietary composition is also important. Gofman *et al.* contend that there is little if any evidence in support of the idea that heart disease in the obese patient is due to the "strain" of the physical burden of carrying body fat. They also point out that if excessive food intake is responsible for the elevated A. I. values, any one of several dietary constituents may be responsible and the problem of delineating the constituent or constituents responsible will be the subject of continuing research. It also must be recognized that different metabolic situations exist during (1) the period of positive caloric balance characterizing the *attainment* of obesity, (2) the period of caloric equilibrium characterizing simply the *maintenance* of obesity, and (3) the period of negative caloric balance, imposed usually by diet, characterizing the *loss* of obesity.

It has been shown in several laboratories (Gofman *et al., loc. cit.*; W. J. Walker *et al., Am. J. Med.* **14,** *654 (1953)*) that reducing diets lower serum lipoproteins and serum cholesterol, but only if these levels are initially elevated. Reducing diets often

contain not only fewer calories, but also less fat, carbohydrate, and even less protein than diets sufficient to maintain weight, and hence many variables are introduced into this experiment.

Gofman and his co-workers (*J. Am. Dietet. Assn., loc. cit.*) have shown that some obese patients placed on low fat but otherwise isocaloric diets will demonstrate a decrease in atherogenic index, and A. Keys (*Am. J. Pub. Health* **43,** *1399 (1953)*) has presented epidemiologic evidence from study of American, Italian and Spanish subjects which he interprets as showing that serum cholesterol is more closely associated with dietary fat than with total calories or the degree of obesity. Dietary cholesterol, in conventional amounts, seems to be completely unrelated to the level of serum cholesterol in man (Keys, *J. Mt. Sinai Hosp.* **20,** *118 (1953)*; *Nutrition Reviews* **12,** *270 (1954)*; M. M. Gertler, S. M. Garn, and P. D. White, *Circulation* **2,** *696 (1950)*).

The effect of caloric balance per se upon serum lipoproteins and cholesterol in the steady state has not been exhaustively studied. Walker *et al.* (*loc. cit.*) showed that force-feeding carbohydrate to 2 normal subjects on a low fat diet for a ten-day period resulted in an increase in weight and an increase in the cholesterol and the lipoproteins of the S_f 12 to 100 range. Although the lipoproteins and cholesterol tended to return toward control values during a second ten-day period of maintenance, the results were equivocal. A longer term experiment with more subjects in which dietary fat and protein are kept constant and in which observations can be made at two levels of obesity, both in the steady state, is needed before the relation of calories per se to serum lipids can be accurately defined.

The amount and quality of protein ingested daily by man may also be a factor of importance in regulating serum lipid levels, and hence may bear upon the course of clinical atherogenesis. Although the effects of protein malnutrition have not been systematically investigated in man, G. V. Mann, S. B. Andrus, A. McNally, and F. J. Stare (*J. Exp. Med.* **98,** *195 (1953)*; *Nutrition Reviews* **12,** *25 (1954)*) have studied this problem in the Cebus monkey. These workers found that a diet high in fat and cholesterol, low in sulfur amino acids and adequate in choline produced marked hypercholesterolemia, elevation of the serum lipoproteins of the S_f 12 to 20 category and typical aortic atheromata. The degree of protein inadequacy was not sufficient to produce any change in total serum protein. The abnormalities in serum lipids and lipoproteins in these monkeys on the amino acid-deficient diet were prevented by inclusion of casein at a level of 25 per cent in place of the deficient protein, and minimized by decreases in total dietary fat or addition of DL-methionine to the deficient diet. The omission of choline from the diet resulted in generally poorer nutrition, absence of hypercholesterolemia and atheromata, and development of fatty livers. This latter finding suggests that grave changes in fat metabolism are without effect upon the course of atherogenesis unless they affect the lipids in the blood compartment of the body.

This observation is also pertinent to the rebuttal offered by Gofman *et al.* (*J. Am. Dietet. Assn., loc. cit.*) to those who deprecate attempts at dietary prevention or management of atherosclerosis "because the body is capable of synthesizing fats and cholesterol from such small molecule precursors as acetate or other two-carbon fragments." Gofman points out that the data available thus far strongly suggest that it is the serum lipid and lipoprotein *content* rather than the turnover rate of these lipids or the content or turnover rate of lipids in tissues other than the blood that bears on the process of atherogenesis. Arguments relative to lipid metabolism in general or to the effect of diet upon lipid metabolism appear, thus, to be irrelevant to the problem of atherosclerosis unless it can be shown that the metabolic

process under consideration influences the blood cholesterol or lipoprotein levels in the steady state.

The use of diet as a preventive or therapeutic tool in the management of atherosclerosis and its complications is suggested by the researches thus far accomplished. It is important that they be continued in order to give better definition to the scientific rationale and the clinical limitations of the nutritional approach to the control of arteriosclerosis.

URINARY AMINO ACIDS AFTER BURNS OR SURGERY

That there is increased nitrogen excretion after injury is well known, but specific urinary nitrogen compounds have not been extensively investigated due, in part, to lack of adequate methods. As discussed previously (*Nutrition Reviews* **3,** *223 (1945)*); **10,** *195 (1952)*), the possibility that a disturbance in the metabolism of one or more amino acids is a factor in the pathogenesis of the increased urinary excretion of nitrogen following injury was suggested a number of years ago, but no conclusive data substantiating this viewpoint have been presented. G. L. Nardi (*J. Clin. Invest.* **33,** *847 (1954)*) has recently applied the technic of paper chromatography to study the urinary excretion of amino acids in 6 patients with severe burns, 5 patients undergoing conventional operative procedures for nontraumatic surgical problems and 3 patients with Cushing's disease. The surgical patients are also reported on by Nardi in *Surgery* **35,** *378 (1954)*.

Aliquots of twenty-four-hour urine collections were analyzed for nitrogen, and volumes corresponding to 250 micrograms of total nitrogen were chromatographed two-dimensionally. The urine samples were not hydrolyzed prior to analysis. Nardi found mainly glycine and alanine in the urine of 5 normal adults with only slight amounts of other "nonessential" amino acids. Occasionally, small quantities of one or more essential amino acids were found. It should be pointed out that considerably more amino acids (including certain of the "essential" amino acids) are found in the urine of normal subjects analyzed by the more precise technic of ion-exchange column chromatography (W. H. Stein, *J. Biol. Chem.* **201,** *45 (1953)*).

Six patients with burns involving from 15 per cent to 65 per cent of their body surfaces were studied. All were presumably in good general health prior to their injury. The patients ranged in age from 19 months to 64 years. The 2 oldest patients (45 and 64 years) were females, were the most extensively burned (50 per cent and 64 per cent of the body surface respectively), and died seven to ten days after injury. The 4 other patients were males and all survived. The burns were of moderate severity in 2 of these patients and very severe in the other 2.

The general treatment was similar for each patient: plasma and whole blood were used for colloid replacement; the burn areas were treated with occlusive dressings; penicillin and streptomycin were given to all. No details of dietary intake are given. Four of the 6 patients with burns were studied over periods of five to ten days after injury; 2 were followed up to one hundred eight and one hundred forty-one days respectively.

Nardi observed an apparent increase in the number of amino acids excreted by the burned patients. This increase began in the first days after injury and was roughly proportional to the severity of the burn. In addition to glycine and alanine, serine, aspartic acid, histidine, tyrosine, glutamic acid, glutamine, taurine, arginine, threonine, lysine, valine, leucine and methionine were all found at one time or another in the urine. Nardi points out that the increased urinary output of amino acid did not necessarily coincide with the period of greatest negative

nitrogen balance, but aminoaciduria in some cases extended beyond the period of negative nitrogen balance, suggesting that the quantitative and qualitative excretion of amino acids was independent of the over-all nitrogen balance. However, no data of total urinary nitrogen or amino acid outputs are given. Nitrogen balance data only are charted; the intake and output data are not reported.

Nardi also reports observations on 5 patients who had undergone the following elective surgical procedures: excision of anal fissure, hemorrhoidectomy, inguinal herniorrhaphy, total hysterectomy and subtotal gastrectomy. All patients were in good nutritional state prior to operation and preoperative urinary amino acid patterns were normal. These patients were studied over periods of two to ten days postoperatively, 3 of them being studied for periods of five days or less. As in the patients with burns, Nardi found increased urinary amino acid excretions roughly parallel to the severity of the surgical procedure, with the individual amino acids assuming an apparently random pattern. The increase in the number of amino acids excreted usually appeared by the first postoperative day and persisted for about a week. Details of dietary intake or total nitrogen output are given. No data are given to as the age or sex of these patients, nor are details of dietary intake or total nitrogen output given. Mention is made that some of these patients, presumably those with the more extensive operative procedures, were on low protein diets for a number of days postoperatively.

Three patients with Cushing's syndrome were also studied. Data on one of these patients, a 42 year old woman, are presented in detail. These patients were studied preoperatively and after undergoing bilateral radical subtotal adrenalectomies. Postoperatively, they were at first given 200 mg. of cortisone daily. This dosage was gradually reduced over a period of two weeks to a maintenance dose of 30 mg. daily. After a number of days on this maintenance level, repeat observations on urinary excretion of amino acids were made. No data are given as to the dietary intake or total nitrogen output of these patients. An initially excessive amino acid excretion was apparently reversed after operation.

In seeking an explanation for the aminoaciduria observed after serious burns or major surgical procedures, Nardi considers the possibilities that the aminoaciduria is a reflection of an increase in the plasma concentrations of the amino acids or consequent to a decrease in renal tubular reabsorption of the amino acids. (Nardi mentions, but quickly discards, the hypothesis of "primary aminoaciduria" as in the de Toni-Fanconi syndrome.) He suggests that elevations of plasma amino acid concentrations might result from increased protein breakdown, increased protein intake, shock, and increased adrenal cortical steroid production. However, he feels that it is unlikely that increased blood concentration of amino acids alone can account for the observed aminoaciduria. He points out that it is doubtful that a high protein intake played a significant role in these patients since the aminoaciduria was subsiding as the dietary intakes were increasing.

There are few data on the plasma levels of individual amino acids after injury or surgical operations. T. C. Everson and M. J. Fritschel (*Surgery* **31,** *226 (1952)*; *Nutrition Reviews* **10,** *195 (1952)*) have reported on the effect of major surgical operations on the plasma levels (determined microbiologically) of arginine, histidine, and the eight amino acids considered essential for man. A transitory fall in the plasma concentrations of all the amino acids immediately after operation was observed. No concomitant increase in urinary excretion of amino acids sufficient to account for the lowering of the plasma levels was observed in the 5 patients in whom such measurements were made. Only the values for threonine, arginine and lysine were still low by the morning of the first postoperative

day. By the third postoperative day, the plasma lysine concentration had risen to normal, while the levels of arginine and threonine rose to normal between the third and seventh postoperative days. No significant increases in plasma levels of any of the amino acids were observed.

Nardi suggests that the aminoaciduria observed after injury is probably due chiefly to renal dysfunction, although no evidence for this has been found. He suggests the possibility that a disorderly, albeit not elevated, pattern of amino acids is presented to the renal tubule which in turn leads to a disturbance of tubular reabsorption. He also suggests the possibility of an increased secretion of an adrenocorticosteroid, probably other than cortisone, which would decrease tubular reabsorption of amino acids. Another possibility is competitive inhibition of tubular reabsorption of amino acids by the presence of compounds in the plasma in higher than normal concentration, for example, creatine. Nardi points out, however, that the increase in creatine concentration necessary for such inhibition is so great that it probably is of no importance.

Nardi has demonstrated an increase in the number and apparent concentration of amino acids in the urine of a small number of severely burned patients and of patients undergoing major surgical operations. It should be emphasized that the analytic technic used by Nardi is not quantitative, nor apparently sensitive, since considerably more amino acids are found in the urine of normal subjects analyzed by ion-exchange column chromatography than were found by Nardi using a paper chromatographic technic. Further, the basic assumption that volumes of urine containing the same amounts of total nitrogen, when chromatographed, can give to the reader results which can be interpreted as to amino acid concentrations is perhaps not warranted, especially since total urinary nitrogen outputs are not given, making estimation of total daily amino acid output impossible. The author also possibly lays too much stress on "essentiality" versus "non-essentiality" as regards the urinary amino acids. The implication that the kidney normally handles the former in a more frugal manner than the latter is not established.

The urinary amino acids are in exchange with the plasma amino acids, which are in turn presumably in exchange with tissue amino acids and tissue and plasma proteins. Studies directed towards correlating these various factors and nitrogen excretion would be helpful.

POTASSIUM ADMINISTRATION TO PATIENTS WITH LOW BLOOD SODIUM LEVELS

The primarily intracellular disposition of potassium and extracellular position of sodium are well known, but their relationship is little known in diseases in which the quantity of one or the other may be altered. Many clinical conditions (for example, diabetic acidosis) are characterized by a loss of potassium from the cells which is replaced to some extent by sodium. It is believed also that patients with edema and with hyponatremia (low blood sodium levels) may have exchanged some potassium for sodium in their cells, thus reducing the extracellular sodium. J. H. Laragh (*J. Clin. Invest.* **33,** *807 (1954)*) has studied the effect of administering potassium orally as potassium chloride to 6 patients with hyponatremia. The patients were one with rheumatic heart disease with edema, one with Laennec's cirrhosis with ascites, another with tetralogy of Fallot (a congenital malformation of the heart) with edema, one patient with tuber-

culous meningitis, and one with hypertension and proteinuria of unknown etiology. Careful balance studies were carried out with 4 of the patients using a sodium restricted diet.

Potassium administration did not induce either a water or a sodium diuresis. In spite of this, significant and sometimes quite striking rises in the serum sodium concentration occurred after a day or two of this regimen. In one instance, for example, the serum sodium concentration which had remained between 115 and 120 milliequivalents (mEq) per liter rose promptly to normal, only to fall again to the previous range after potassium administration was discontinued. The same phenomenon was observed to a greater or lesser degree in the other patients. Rises in the serum potassium concentration also occurred, sometimes to what might be considered dangerous or toxic levels (the highest was 9.5 mEq per liter, rising from 4.5).

The author believes that as the patients were neither ingesting sodium in significant amounts nor excreting increased quantities in the urine when the potassium was administered, and as there were no changes noted in the body fluids or urine volume, the most likely explanation for the phenomenon was that some of the potassium administered was exchanged for sodium in the cells, releasing sodium which was not excreted but served to increase the serum sodium concentration and correct the hyponatremia. Not only was there no increased sodium excretion after potassium administration, but in a similar manner potassium itself did not seem to be excreted so well as in normal individuals, but resulted in hypokalemia (low blood potassium). The possible dangers of potassium administration in this circumstance and the lack of diuretic effect are stressed by the author.

TREATMENT OF NUTRITIONAL MACROCYTIC ANEMIA

A dietary treatment of nutritional macrocytic anemia was described in 1931 by L. Wills (*Brit. Med. J.* **1,** *1059 (1931)*). The condition is characterized by macrocytic anemia, watery diarrhea, edema, and megaloblastic arrest of the bone marrow. It is commonly seen among the poorer economic classes and is usually associated with a history of poor diet. Wills reported that the condition would respond to yeast and crude liver extracts, and this observation eventually led to the discovery of folacin (P. L. Day, *Vitamins and Hormones* **2,** *71 (1944)*). Since pernicious anemia, in which the blood picture is similar to nutritional macrocytic anemia, will respond initially to either folacin or vitamin B_{12} it is desirable to compare the two vitamins in the treatment of the nutritional anemia. The discovery of the citrovorum factor, which may be the active metabolic derivative of folacin, also permits further study of the etiology of nutritional macrocytic anemia.

V. Ramalingaswami and P. S. Menon (*Indian J. Med. Res.* **37,** *471 (1949)*) reported the effectiveness of orally administered folacin in nutritional macrocytic anemia. Six adult patients with typical signs of the disease were selected for the study. All were free of malarial and helminthic infections and demonstrated free hydrochloric acid in their gastric contents, thus ruling out pernicious anemia. The patients were hospitalized and given a diet of rice, vegetable soup, bread, butter, tea and one orange per day. The initial dose of synthetic folacin was 20 mg. daily which was reduced to 10 mg. after from two to three weeks. All 6 patients responded to the treatment. The initial erythrocyte count of 1.67 million cells per microliter had risen to an average of 3.93

after four weeks of treatment. A pronounced reticulocyte response was observed after from six to eleven days of treatment. The longest observation period was three months at which time there was no relapse in the condition. Thus, over the observation periods employed, folacin appeared to cure completely nutritional macrocytic anemia.

In a more recent report, M. S. Narayanan, R. Kalamegham, and V. Ramalingaswami (*Indian J. Med. Sci.* **8,** *131 (1954)*) have studied the effectiveness of citrovorum factor and vitamin B_{12} in the treatment of nutritional macrocytic anemia. Seven adult patients, similar to those used in the folacin studies, were selected. Five of these patients were given a single injection of 24 mg. of synthetic citrovorum factor (leucovorin). There was a typical blood response in all patients. The red cell count and reticulocytes increased and the mean cell volume decreased. Bone marrow studies demonstrated a change from megaloblastic to normoblastic marrow following the injection. This improvement lasted approximately two weeks, after which time the blood picture began to revert to the original condition of macrocytic anemia. It appears that a single dose of citrovorum factor is effective for approximately two weeks in the treatment of nutritional macrocytic anemia. The authors did not determine if these patients in relapse would respond to an additional dose of citrovorum factor.

Two patients with nutritional macrocytic anemia were each given a single dose of 120 micrograms of vitamin B_{12}. These patients gave a hematopoietic response quite similar to that observed in patients receiving citrovorum factor. However when vitamin B_{12} was given the blood picture was still normal thirty-six days after treatment, the longest observation period reported.

In order to evaluate the effectiveness of the various forms of treatment of nutritional macrocytic anemia much longer observation periods must be employed. It is quite possible that the condition responds as does pernicious anemia with folacin, giving an initial response but eventually becoming ineffective. Long-range studies on nutritional macrocytic anemia might be conducted to advantage to aid understanding of the effectiveness of the antianemic factors.

HUMAN GROWTH

Growth is the resultant of genetic and environmental factors, including nutrition. The hereditary mechanisms in growth are difficult to study in man. Consequently, the human nutritionist will examine with interest G. E. Dickerson's survey of the sources of variation in animal growth ("Dynamics of Growth Processes," edited by E. J. Boell, *p. 242, Princeton University Press (1954)*). The animal geneticist has the great advantage that his practical goals are well defined and his success is readily measurable, as when he deals with improving the efficiency of domestic animals as converters of grain and forage into meat. For man the effects of different rates of growth, as measured in terms of longevity and health during maturity, are the basic criteria. For various reasons they have not been adequately examined up to this time, but relevant data are being accumulated at several research centers.

The paths through which the genes affect growth are complex and include effects on specific nutrient requirements, on endocrine activity, and on sensitivity of response to hormones. Genetic variation in requirements for specific nutrients has been shown to be an important source of heritable variation in the growth of animals.

In addition to general size and gross weight, the genetic control of the growth of particular portions or tissues of the body is of interest. Thus G. E. Dickerson and J. W.

Gowen (*Science* **105,** *496 (1947)*) observed that the gene associated with the yellow coat color in the mouse caused a continued accumulation of large amounts of fatty tissue after the growth of the nonyellow litter mates had plateaued. At the same time there were no detectable differences in the growth of other tissues. The "yellow" gene accomplished the increased fat deposition (and a lowered food requirement per unit of weight gain) by slightly increasing the appetite and by reducing the energy spent in the activity of the body.

In the wider context of studies on growth the dietary factors in growth and the problem of assessment of individual growth were briefly considered by L. W. Sontag and S. M. Garn (*Ann. Rev. Physiol.* **16,** *37 (1954)*). It has been shown in laboratory animals that dietary deficiencies, experimentally induced during pregnancy, result in a variety of developmental defects in the offspring such as hydrocephaly, cleft palate and skeletal defects. Sontag and Garn believe that some of the developmental abnormalities noted in human births must be attributable to vitamin deficiencies and maternal hypoproteinemia. However, assembling adequate evidence is a difficult task. It is pointed out that women who are conscientious and able to report regularly and carefully on their diets are likely to be careful also about what they eat; this reduces the range within which the "independent variable" (dietary intake) will vary and crucial comparisons are difficult to make. On the other hand, women indulging in dietary fads and fancies likely to result in dietary deficiencies are likely to distort the food consumption records. A careful study by B. S. Burke, V. A. Beal, S. B. Kirkwood, and H. C. Stuart (*J. Nutrition* **26,** *569 (1943)*) has substantiated the results obtained in rigorously controlled animal experiments, demonstrating a statistically significant relationship between the diet of the mother during pregnancy and the condition of her infant at birth and within the first two weeks of life. In the 216 cases considered in this study all the stillborn infants, all but one infant who died within a few days after birth, most of the infants with marked congenital defects, all premature infants, and all functionally immature infants were born to mothers whose diets during pregnancy were considered as very inadequate.

Sontag and Garn devoted some attention to the technics for the description and evaluation of human growth, a topic discussed in detail but in a narrower framework by W. M. Krogman ("A Handbook of the Measurement and Interpretation of Height and Weight in the Growing Child," *Monograph Soc. Research Child Develop. No. 48 (1950)*). In the opinion of Sontag and Garn (*loc. cit.*) there is no satisfactory answer to the important medical-nutritional question regarding the degree of conformity to a norm to be expected on the part of an otherwise healthy child.

A schedule for routine assessment of nutritional status of Indian children was described and examined by K. S. Rao, A. D. Taskar, and M. K. Ramanathan (*Indian J. Med. Res.* **42,** *55 (1954)*). They were concerned with the somatometric data rather than the clinical signs of diseases associated with nutritional defects. The measurements included height, weight, hip width (intercristal diameter), and 5 skinfolds. The age and sex differences in hemoglobin level are also of interest. The measurements were made on boys 2 to 19 years old, and on girls in the age range of 2 to 14 years. The number of children at different age levels was quite uneven, varying from 15 to 150 for boys, from 6 to 81 for girls. The total sample included some 2300 children from the Nilgiri district. The measurements of hip width and of the amounts of subcutaneous tissue were made on a smaller number of children. For the skinfolds only the mean values were given while for the other measurements a measure of dispersion (standard deviations) was also provided.

There are apparently no valid Indian

norms of average changes in height, weight and hip width with age. This makes it difficult to evaluate, on the basis of the bodily measurements, the development of children studied by Rao *et al.* It was noted that the children in the area in general looked thin and undersized, with poor musculature and deficient subcutaneous adipose tissue. The children included in the study belonged mainly to poor and lower middle-class families and there were frequent clinical signs of ill health (enlarged tonsils, chronic rhinitis (inflammation of the mucous membranes of the nose), infections of the skin, respiratory infections and diarrhea). Thus the data obtained on this sample cannot provide the needed growth norms and, as indicated above, are uninterpretable because of the lack of proper control measurements on healthy children. Establishment of proper norms should be the first concern in the application of nutritional anthropometry.

In the evaluation of bony development an important role is played by establishing the appearance of centers of ossification (*cf.* W. W. Greulich and S. I. Pyle, "Radiographic Atlas of Skeletal Development of the Hand and the Wrist," *Stanford University Press (1950)*; for skeletal development in infancy, see E. L. Reynolds and T. Asakawa, *Am. J. Roentgenol.* **65,** *403 (1951)*). A method based on a large number of bones was described and normative data were given by V. S. V. Harding (*Child Development* **23,** *247 (1952)*). This work is based on x-rays collected in the course of a longitudinal study carried on since 1930 in the Department of Maternal and Child Health, Harvard University School of Public Health, under the direction of H. C. Stuart. Some 50,000 x-ray films of 323 children were examined in collecting the basic data.

For different age levels (birth to 16 years) the percentages of children in whom each of the forty-odd osseous centers have been observed are tabulated. This has been done separately for the two sexes, as the girls are ahead of the boys in the appearance of osseous centers. The range of ages within which each center has been observed to appear in the middle 50 and the middle 80 per cent of the children is also given. Thus for the patella the 50 per cent range in the time of appearance of the center of ossification is 3 years 5 months to 4 years 11 months of age, the 80 per cent range from 2 years 11 months to 5 years 10 months. Technically, these values correspond to the 25th and the 75th, and the 10th and 90th percentiles, which may be used as reference points for judging the "bone age" as retarded, normal, or advanced.

For the evaluation of the osseous development of a given child and its bony age a chart was prepared in which at each age level a series of centers is to be checked, indicating the range from retardation to accelerated development. The chart is particularly useful for periodic examinations. It was noted that children tend to maintain a fairly constant rate of osseous development over long periods of time. Nutritional histories of these children are on file. It will be of interest to examine the relations between the dietary data and the appearance of ossification centers.

The value and limitations of information on the skeletal status in the framework of appraising the physical growth and development of children were considered in some detail by Greulich ("Dynamics of Growth Processes," *p. 212, loc. cit.*). He limited his attention to the x-ray films of the child's hand and the wrist, considered as a dependable indicator of the body's general level of maturity which, at the same time, is fairly independent of bodily size. Both the occurrence of ossification centers and the obliteration of the cartilages joining the ends of long bones (epiphyses) to their shafts are considered as indicators of the skeletal age. In healthy individuals the skeleton tends to develop as a unit, with the various parts keeping pace with each other in their maturation, and the status of the hand and wrist is considered as indicative of the maturative changes in the skeletal system as a whole. The technic for the determination of a

child's skeletal age may be employed from birth to the completion of skeletal development during early adulthood.

The height-weight-age tables have inherent limitations as a criterion of "nutritional status" to be used with adults. The same relative weight at different ages may represent quite different degrees of "fatness" (*cf.* J. Brozek and A. Keys, *Science* **112,** *788 (1950)*). In children important additional limitations are added in using weight and height as criteria of physical development. Greulich points out that the population of the United States is very heterogeneous in national and racial origin. Even among the whites there are such diverse groups as the short-statured Mediterranean people and the taller and heavier Scandinavians. This genetic diversity in the average ultimate (adult) stature is further complicated by the existence of early and late maturing strains in the population. With wide differences in the age of the onset of puberty and the associated preadolescent spurt of growth, the chronologic age of the child during the early part of the second decade of life is an unreliable reference point for evaluating his developmental status. It is not a good indicator of the amount of progress that the child should have made toward attaining adulthood. These difficulties, among other factors, stimulated interest in the use of the status of the skeleton as an index of physical development.

Growth and development proceed, in general, in parallel, but they represent different and potentially independent processes. Growth manifests itself by an increase in mass and volume as disclosed by an increase in weight and by changes in external dimensions. Development refers to the child's progress toward maturity. The x-ray analysis of the wrist and hand is a useful index of the developmental status because of the correspondence between the maturation of the skeletal and the reproductive systems. Greulich ("Dynamics of Growth Processes," *loc. cit.*) illustrates this correspondence on a case of precocious puberty in which there was an abnormal acceleration of the skeletal development, as indicated by the presence of ossification centers and the advanced degree of fusion of the epiphyses of the finger bones. The epiphyses are pieces of bone at the end of longer bones which in early life are separated from the latter by cartilage and later become a part of the larger bone. At this time the growth of the long bones ceases. In individuals with gonadal inadequacies and retarded sexual development the growth, especially in the extremities, continues for an abnormally long period, resulting in long limbs and short trunk. In normal girls the maximal annual increment in height takes place during the year preceding that in which the first menstruation occurs, regardless of the chronologic age.

In addition to the appearance of ossification centers and the fusion of the epiphyses, the growing ends of the shafts of the long bones may bear the mark of illness and other adverse influences, including malnutrition. Reference to children studied at the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, is of particular interest here. The children, when first observed, were retarded in growth and their bones were poorly mineralized. It appears that following the addition of reconstituted dry milk to their diet the mineral materials began to be deposited in greater concentrations than before, resulting in transverse lines of increased density in the roentgenogram of the lower arm (the radius). The increased rate of deposition preceded, presumably, the increased rate of bone growth. When an equilibrium was subsequently established between the rate of growth and mineral deposition, the new bone assumed a normal, homogeneous appearance. It was somewhat denser than the bone laid down before the dietary supplementation.

The relationships among nutrition, growth and development are complex. Genetic endowment provides the limits within which nutritional factors can operate and the endocrine system is importantly involved. The x-ray analysis makes it possible (1) to

distinguish the poorly from the adequately mineralized skeleton, and (2) to judge skeletal age from the presence or absence of ossification centers and epiphyseal fusions, as a useful measure of general body maturity. Thus it provides an important research tool and a useful supplement to the clinical evaluation of nutritional status.

PARENTERAL AUREOMYCIN AS GROWTH FACTOR FOR CALVES

In a preliminary study workers at the Louisiana Agricultural Experiment Station, Baton Rouge, observed that aureomycin stimulated the growth of young calves when it was administered by intramuscular injection (L. L. Rusoff, J. M. Fussell, C. E. Hyde, and R. M. Crown, *J. Dairy Sci.* **36,** *593 (1953)*). A more extensive study confirming this earlier observation has recently appeared (Rusoff and co-workers, *Ibid.* **37,** *488 (1954)*).

Three groups consisting of 8 male Jersey calves each were subjected to the following treatments: group 1 served as control, the group 2 animals each received weekly injections of 400 mg. of aureomycin as an aluminum chloride complex in sesame oil, and the group 3 animals received an equivalent amount of the antibiotic by mouth.

All calves received colostrum the first three days after birth before they were placed on experiment. For the first thirty days of the experiment milk was fed at the rate of 1 pound per 10 pounds of body weight. A calf starter and clover hay were offered ad libitum from the eighth day. The calf starter had the following percentage composition: cottonseed meal, 35; soybean oil meal, 5.5; yellow corn meal, 31.5; crimped oats, 15; wheat bran, 10; steamed bone meal, 2; salt, 1. Vitamins A and D were supplied in the starter at the rate of 20,000 and 5000 units respectively per kilogram of diet.

The control calves gained an average of 0.78 pounds daily during the sixteen-week experimental period. The calves receiving injections of aureomycin averaged 1.07 pounds each in daily body weight gain while those receiving the antibiotic orally gained 0.94 pounds daily. The significantly better weight gains due to the antibiotic were associated with more efficient utilization of the feed (calf starter) for growth.

The animals receiving the antibiotic also were observed to have gained significantly better than the control calves when height at withers served as the criterion of response. Thus, a height increase of 5.99 inches was observed during the experiment in control animals, contrasted with height increases of 7.59 and 6.44 inches for animals receiving the antibiotic intramuscularly and orally. Since this measurement reflects the growth of skeletal tissue, the authors suggest that the antibiotic may be exerting its beneficial effects on growth through an influence on bone metabolism.

Rumen studies on the control, aureomycin-injected, and aureomycin-fed calves indicated that the antibiotic had no significant effect on rumen microorganisms as measured by fiber digestion by rumen contents under *in vitro* conditions. There were likewise no significant changes in the concentration of B-vitamins in the rumen contents of control and antibiotic-treated calves.

The experiment also included an attempt to establish the pathway of excretion of the intramuscularly injected and orally administered antibiotic. Based on concentration of aureomycin found in urine and feces and in bile at autopsy it was evident that the principal pathway of elimination, following injection, was via bile and urine; after oral administration the level in feces was much greater than that in urine. From these results it was considered a reasonable assumption that injected aureomycin in ruminants probably by-passes the rumen, although the antibiotic concentration in the

rumen was not determined in any of the animals.

In attempting to explain a growth stimulation from antibiotics in ruminants the hypothesis has been advanced that the effect may be due to changes in the microbial populations in the rumen. The Louisiana workers, basing their premise on failure to detect any rumen alterations, prefer to explain their results as indicative of a direct stimulatory action of the antibiotic on the body tissue, possibly such as an increase in bone deposition. The marked stimulation observed from injected antibiotic tends to support this explanation.

The possibility that mineral absorption and utilization may be favorably influenced by antibiotics has been suggested by others. B. B. Migicovsky, A. M. Nielson, M. Gluck, and R. Burgess (*Arch Biochem. Biophys.* **34,** *479 (1951)*) reported that penicillin enhanced the absorption of calcium in baby chicks. Bone calcification under suboptimum vitamin D intake was improved with penicillin (E. Ross and H. Yacowitz, *Poultry Sci.* **31,** *933 (1952)*). A lowered manganese requirement for the prevention of perosis in chicks was attributed to aureomycin by W. F. Pepper, S. J. Slinger, and I. Motzok (*Ibid.* **30,** *926 (1951)*).

Poultry nutrition researchers have repeatedly observed that chicks will often not respond to antibiotic feed supplements when they are grown in new quarters where chickens had not been housed previously. Such results have been interpreted as evidence that a "disease level" establishes itself in quarters where chicks are habitually housed and that antibiotics in the feed serve to reduce the disease hazard.

In a recent report from the Kansas Agricultural Experiment Station a comparison was made of the effect of old versus new quarters on growth stimulation from antibiotics in dairy calves (E. E. Bartley, F. W. Atkeson, H. C. Fryer, and F. C. Fountaine, *J. Dairy Sci.* **37,** *259 (1954)*). These workers observed that in new quarters, in which sickness was not a great problem, calves fed no aureomycin grew as rapidly as calves fed aureomycin in old contaminated quarters. However, a further growth stimulation was noted when calves in the new quarters received the antibiotic in their feed.

These and other recent reports, designed to elucidate the mode of action of antibiotics in stimulating animal growth, again serve to illustrate that this problem continues to challenge the imagination of the nutrition investigator.

PROTEINS IN THE NUTRITION OF RUMINANTS

It is widely recognized that rumen microorganisms contribute abundantly to the supply of protein digested in the lower regions of the digestive system. It is for this reason that the ruminant animals (cattle, sheep and goats) are less susceptible to critical deficiencies of individual amino acids in their food protein than are animals such as the rat, pig, chicken, or man. One consequence of this fact has been to take advantage in animal maintenance of the synthetic powers of rumen organisms and to obtain high quality microbial protein from nonprotein nitrogenous compounds such as urea.

Out of such experiments it has become evident that the value of urea or other simple nitrogen compounds as sources of microbial protein is partly dependent upon the rapidity with which the urea is converted to ammonia. If this process proceeds rapidly, much of the urea nitrogen may be converted to ammonia and lost via the kidneys (*Nutrition Reviews* **12,** *43 (1954)*).

Several reports have appeared in the British literature which provide evidence

that the value of proteins for ruminants may vary inversely with the extent to which the protein is attacked and deaminated by rumen microorganisms. R. M. Pearson and J. A. B. Smith (*Biochem. J.* **37,** *153 (1943)*) observed rapid degradation of casein and gelatin by rumen organisms (rumen liquor) *in vitro*. I. W. McDonald (*Ibid.* **51,** *86 (1952)*) established *in vivo* in sheep that ammonia was an important product in the degradation and that different proteins were degraded at different rates. More recently McDonald (*Ibid.* **56,** *120 (1954)*) reported that the nitrogen of zein (corn protein) is converted very slowly to ammonia.

The rapid conversion of casein nitrogen to ammonia in the rumen and consequent loss via the kidneys was recently demonstrated by M. I. Chalmers, D. P. Cuthbertson, and R. L. M. Synge (*J. Agr. Sci.* **44,** *254 (1954)*). They observed that casein supplements when fed to pregnant ewes on a relatively poor plane of nutrition (negative nitrogen balance) was ineffective in bringing about retention of nitrogen.

With fistulated steers the following experiment was conducted. During a basal eight-day period 6.06 g. of nitrogen were fed daily with a urinary output of 6.82 g. and a fecal output of 2.78 g. of nitrogen. Supplying an additional 6.15 g. of nitrogen as casein via rumen fistula resulted in 11.42 g. urinary and 3.16 g. of fecal nitrogen output daily during a seven-day period. This represented a very low retention of the supplementary nitrogen. During the subsequent twelve-day period the 6.15 g. of nitrogen as casein was introduced via duodenal fistula so that the rumen was bypassed.

The urinary nitrogen excretion decreased to an average of 9.44 g., while the fecal nitrogen excretion remained the same. This represented a considerably improved nitrogen retention from supplementary casein and suggested that the rumen organisms were probably responsible for poor nitrogen retention because of amino acid deamination and consequent loss of ammonia via the kidneys.

In vitro experiments were conducted to establish that as much as 2.4 g. of casein nitrogen out of 13 g. in the casein fed were converted to ammonia within five hours by rumen liquor. This rapid ammonia production *in vitro* could be reduced considerably by substituting the ordinary commercial casein with a hardened casein (treating commercial casein with normal aqueous sodium hydroxide and 2-normal lactic acid, water washing, and drying at 105° C. to produce a horny material). The same hardened casein, when fed as the supplement in nitrogen balance studies, was found to be considerably better retained than the untreated commercial casein.

It is of interest that the treatment described above which improved the casein for sheep, as evidenced by improved nitrogen balance, is well known to impair the value of casein for animals with simple stomachs such as rats and dogs.

In another study these workers compared casein with herring meal as a protein supplement for sheep (M. I. Chalmers and R. L. M. Synge, *J. Agr. Sci.* **44,** *263 (1954)*). These studies were intended to establish whether the extent of ammonia formation in the rumen would be reflected in weight gains and nitrogen retention alteration commensurate with the theory that excess ammonia formation is representative of poor protein utilization. It was found that casein consistently caused a greater rise in ammonia in rumen liquor than did herring meal. Under the same dietary conditions, casein nitrogen was not retained so well as herring meal nitrogen. Furthermore, it was found that on diets that were limiting in protein the herring meal supplement produced better weight gains than the casein when fed to growing lambs. These results established that the degree of ammonia formation under different dietary protein

supplementations could serve as a prediction of the value of different feed proteins for sheep.

In another study the relation of other dietary constituents to the concentration of ammonia in rumen liquor was investigated (M. I. Chalmers, R. L. M. Synge, E. F. Annison, and S. B. M. Marshall, *J. Agr. Sci.* **44**, *270 (1954)*). Protein sources in these experiments were peanut (ground nut) meal, corn gluten meal, and herring meal. The rumen ammonia concentration that resulted when peanut meal was fed was high, equal to or greater than that found for commercial casein, and indicative of rapid deamination. In confirmation of the earlier studies by McDonald (*loc. cit.*), corn gluten meal produced practically no increase in ammonia in the rumen liquor.

The presence of liberal amounts of readily fermentable carbohydrate in the diet (cereal meal or starch) was effective in reducing the ammonia production when protein-rich material was introduced into the rumen. This is consistent with the idea that energy-rich material stimulates the multiplication of rumen organisms that can rapidly assimilate amino acids arising from the degradation of protein in the rumen.

These results raise the question of the reliability of digestibility data as criteria for assessing the value of proteins for ruminants. Although the reliability of digestibility data alone, as an index of protein quality, has been challenged by others, the present results have established a reasonable explanation for the differences in value of protein sources for ruminants.

One of the questions raised by the British workers is the possible misinterpretation or possible false conclusions that may have been drawn from experiments in which the value of urea as a protein replacement for ruminants was determined by using casein as the reference protein. These workers feel that such experiments have presented unduly favorable impressions of the usefulness of urea. It is unfortunate that the British workers did not include urea as a nitrogen source in their rumen ammonia concentration and nitrogen balance studies.

SULFUR-METHIONINE-SELENIUM RELATIONSHIPS IN CHLORELLA

The toxicity for plants of seleniferous (high selenium) soils can be overcome by the addition of sulfate to the soil (A. M. Hurd-Karrer, *Am. J. Bot.* **24**, *720 (1937)*; **25**, *666 (1938)*). Analyses of plant tissues indicated that an increase in the sulfate supply decreased selenium absorption (S. F. Trelease and O. A. Beath, "Selenium," *published by the authors, New York (1949)*). The toxicity of selenium apparently involves an interference with sulfate metabolism in plants, and it is believed that selenium can be metabolized to some degree by the same enzyme systems that reduce sulfate. In this way it is presumed that the selenium analogues of the sulfur amino acids could be formed in plants and these would likewise contribute to toxicity symptoms. Although the selenium analogues of cystine or methionine have not been isolated from plants, amino acid complexes containing both sulfur and selenium have been reported (M. J. Horn and D. B. Jones, *J. Biol. Chem.* **139**, *649 (1941)*).

The interesting, and as yet not clearly understood, sulfur amino acid-selenium relationship in plants was recently further investigated by A. Shrift (*Am. J. Bot.* **41**, *345 (1954)*). Stock cultures of *Chlorella vulgaris* served as the test organism. The organism was grown on dextrose-agar with added inorganic salts.

The activity of the selenium analogue of methionine (DL-seleno-methionine) was

tested in the presence of sulfate. At concentrations as low as 0.31 parts per million (p.p.m.) of selenium, a lag occurred in the growth of the organism when the medium contained 10 p.p.m. of sulfur as potassium sulfate. With increasing amounts of the DL-seleno-methionine the lag phase was extended and the rate of growth was also decreased. Additions of increasing amounts of sulfate did not overcome the lag in growth response that was induced by seleno-methionine. One unusual feature of the growth observed in the presence of seleno-methionine was an apparent inhibition of cell division and a marked increase in the size of the existing cells. These cells assumed sizes two times the diameter of normal cells.

Cells that emerged from the lag phase were capable of growing in the presence of seleno-methionine without exhibiting the lag phase. This adaptive response indicated that a detoxification mechanism had been evolved during the initial lag phase either by way of increased methionine synthesis or by development of enzyme systems capable of inactivating the seleno-methionine.

The failure of sulfate to aid or overcome the lag phase led to a test of the value of reduced sulfur in the form of methionine as a counteractant. With L-methionine present at a level of 10 p.p.m. of sulfur, increasing amounts of seleno-methionine decreased growth rate and final population. Increasing the L-methionine concentration again restored growth approaching that observed in the absence of seleno-methionine. Simultaneous increase of the L-methionine and the seleno-methionine, while a S/Se ratio of 1 was maintained, resulted in uniform growth. These results established that the seleno-methionine was a competitive analogue for L-methionine, but that inorganic sulfate was not able to overcome the effects of seleno-methionine.

Further tests were conducted to establish the ability of D-methionine to counteract the toxicity of seleno-methionine. The growth responses obtained in the presence of seleno-methionine and D-methionine were similar to those observed with the selenium compound in the presence of sulfate. With higher concentrations of the selenium compound cell division was interrupted and giant cells resulted during the prolonged lag. Although the D-form of methionine was able to elicit improved growth with increased concentrations in the presence of seleno-methionine, it did not exhibit the antagonist reaction that could be considered competitive, as was observed with L-methionine.

These observations led the author to the following analysis of selenium-sulfate-methionine relationships in *Chlorella*: (1) L-methionine is a true competitive antagonist for seleno-methionine and the competition is evidenced by a reduced absorption of the seleno-methionine analogue in the presence of L-methionine. (2) Sulfate and selenate are also a true competitive pair. (3) In both above instances the competition appeared to involve two points of action, namely, competition for absorption sites at the cell membrane and competition within the cell for an enzyme responsible for the utilization of the metabolite.

In terms of plant nutrition the author pictures these relationships somewhat as follows. Selenate inhibits the reduction of sulfate for the synthesis of L-methionine in the plant. D-seleno-methionine competes with D-methionine, decreasing the rate of synthesis of L-methionine in the plant and exhibiting growth depression in this manner. L-seleno-methionine competes with L-methionine in the enzyme system transforming L-methionine in a reaction involved in cell division, an explanation for the presence of giant cells in such systems. The last suggested relationship is in harmony with the explanation offered by M. N. Camien and M. S. Dunn (*J. Biol. Chem.* **184**, *283* (*1950*)) to account for the interference of DL-ethionine in the utilization of D- and L-methionine by lactic acid bacteria.

It has been postulated that selenium might replace sulfur in the amino acids of the proteins in animals. A. L. Moxon, K. P. DuBois, and R. L. Potter (*J. Pharmacol. Exp. Therap.* **72,** *184 (1941)*) found that the L-cystine selenium analogue was as toxic for animals as sodium selenite for seleniferous wheat. The D-cystine selenium analogue was far less toxic than the L-form, implying a lesser competitive effect of the unnatural isomer.

Evidence that selenium is incorporated into sulfur amino acids in man was established indirectly in the treatment of patients suffering from selenium poisoning (R. E. Lemley, *Journal-Lancet* **60,** *528 (1940)*). It was noted that brombenzene, given orally in small amounts, resulted in a sharp increase in the urinary excretion of selenium and the selenium excreted was in the mercapturic acid fraction of the urine. This is an example of utilizing a detoxification mechanism in reverse. Normally, animals detoxify brombenzene by conjugation with a sulfur amino acid to form mercapturic acid. In Lemley's treatment the brombenzene was evidently used to detoxify the toxic selenium analogue of cystine. The apparent similarity between plants and animals regarding the selenium, sulfate, and sulfur amino acid relationships raises the question of possible effects of arsenic in plants. Arsenic, when given to animals (rats), protects the animals against selenium poisoning. Whether or not this effect is mediated through suppression of seleno-methionine synthesis has not been determined.

HEMOGLOBIN AND BODY IRON IN PROTEIN DEFICIENCY

When the diet is relatively low in protein there must be competition for the available protein by the various body tissues. The studies of G. H. Whipple (*Am. J. Med. Sci.* **203,** *477 (1942)*) and his co-workers (Whipple, L. L. Miller, and F. S. Robscheit-Robbins, *J. Exp. Med.* **85,** *277 (1947)*) indicated that hemoglobin had a rather high priority when the anemia was produced by repeated hemorrhage. Although there have been many studies on the anemia produced by protein or specific amino acid deficiencies, few investigations on total hemoglobin synthesis are available. J. Metcoff, C. B. Favour, and F. J. Stare (*J. Clin. Invest.* **24,** *82 (1945)*) found that although the hemoglobin concentration was not decreased by feeding an 8 per cent protein diet to rats for ten to fourteen weeks, the total circulating hemoglobin, blood volume, and red cells per unit body surface were lowered. On the other hand, a slight anemia (drop in hemoglobin concentration) was produced by feeding a 3.5 per cent protein diet (A. U. Orten and J. M. Orten, *J. Nutrition* **26,** *21 (1943)*) which was shown by controlled feeding not to be due to the coincident lower food intake. No studies of simple protein deficiency seem to be available on human beings. In the well-known Minnesota Experiment on partial starvation (A. Keys *et al.*, "The Biology of Human Starvation," *Univ. of Minnesota Press, Minneapolis (1950)*) anemia occurred. The fall in calculated total hemoglobin was slightly greater than the decrease in body weight but seemed to be about parallel to the decrease in active tissue.

The effect of protein deficiency upon iron absorption and metabolism and its relation to the resultant anemia has not been previously studied. B. Hallgren (*Acta societatis medicorum Upsaliensis* **59,** *79 (1953)*) has published extensive findings on rats fed various levels of iron and with diets adequate or deficient in protein. In addition to the usual blood studies, the total body hemoglobin was determined by viviperfusion with a modified Tyrode solution in a manner similar to that described by D. H

Copp and D. M. Greenberg (*J. Biol. Chem.* **164,** *377 (1946)*), except that the perfusion was made through the femoral vein. Studies of the tissues after perfusion indicated that only 2 to 3 per cent of the hemoglobin remained in the tissues, mostly in the bone marrow and spleen. Nonhemin iron was determined by extraction of the tissues with 5 normal hydrochloric acid for one hour at 85 to 90° C., a procedure which appears to liberate insignificant amounts of iron from hemin. Plasma iron, total body dry weight, protein and fat were also determined. The studies were done using a diet of the purified type in which heated egg white supplied the protein. It contained approximately 0.25 mg. of iron per 100 g. of diet. The author states that this is about one-fifth the amount in cow's milk on a caloric basis. When iron supplements were given, these were added daily to the diets as ferrous sulfate.

In the first experiment young adult male rats were fed the purified diet containing 18, 9, 4.5, or 2.25 per cent of protein as dry egg white. The first three groups received 18 g. of feed daily while the last group failed to eat this amount. The animals were killed after approximately one hundred days except for the last group which were killed after sixty days when it appeared likely that they would die of protein deficiency. Although the first two groups (18 and 9 per cent protein) were similar and probably not significantly different, both the hemoglobin concentration and the total body hemoglobin fell with protein deficiency of increasing severity. At the 2.25 per cent protein level the hemoglobin concentration fell to 85 per cent of the control group and the total hemoglobin fell to 60 per cent of the control. Since the protein in the carcass fell to about 70 per cent of the high protein group, the total hemoglobin per gram of body protein was only moderately decreased, *i.e.*, about 14 per cent. It is of interest that as the hemoglobin fell the nonhemin iron rose so that the average body iron of all groups was approximately 11 to 12 mg. Although there may be some disagreement on the basis of comparison, the author concludes that there was no evidence of priority of hemoglobin synthesis in these studies. He was impressed by the observation that the adrenals were smaller in the deficient animals, and this was studied further.

In the next experiment young rats were fed either 22 or 9 per cent protein diets. In order to keep the animals on the low protein diet clearly deficient, the level of protein was decreased to 8 per cent at forty-five days, 4.5 per cent at sixty-eight days, and finally to 4 per cent at eighty-two days. Both the protein-sufficient and protein-deficient groups were subdivided into subgroups which received the following daily iron supplements: 0, 35, 70, 280, and 560 micrograms. The animals on the high protein diet were killed after approximately sixty days while those restricted in protein were killed after about one hundred to one hundred thirty days. Raising the level of iron from 280 to 560 micrograms per day produced only slight effects on either the low or high protein diet. Combining the data at these levels of iron provides a comparison of the effects of the two protein levels at adequate iron intake. The average body weights and total body protein of the high and low protein groups were 198 and 23.5 g. compared to 185 and 22.8 g. respectively, not greatly different; however, the protein-deficient group had a slight anemia. The hemoglobin concentration was 14.7 per cent, red cell count 7.7 million per cubic millimeter, hematocrit 42 per cent, mean corpuscular volume 55 cubic microns, and mean corpuscular hemoglobin 19 micromicrograms. Corresponding values from the high protein diet were 16.4 per cent, 6.7 million, 46 per cent, 69 cubic microns, and 25 micromicrograms. The total body hemoglobin was 1.55 and 1.18 g. for the high and low protein groups respectively. Plasma iron was 216 and 140 micrograms per cent and adrenal weights

were 35 and 24.8 mg. The protein deficiency anemia was thus hypochromic and microcytic.

Although the blood picture and hemoglobin were not affected by raising the iron intake from 280 to 560 micrograms per day, the nonhemin iron of the liver and spleen increased while the nonhemin iron of the remaining carcass was not significantly increased. However, the main observation was that in spite of the anemia the nonhemin iron of the liver and spleen of the protein-deficient animals was much higher than that of the control animals. At the same level of iron intake, 560 micrograms per day, the livers of the deficient group contained 13 mg. per 100 g. and the spleen 46 mg. per 100 g. compared to 3.2 and 22 mg. respectively for the high protein group. The absorption of iron is believed to be normally controlled by a "mucosal block" (S. Granick, *Ann. N. Y. Acad. Sci.* **48,** *657 (1947)*). C. J. Gubler, G. E. Cartwright, and M. M. Wintrobe (*J. Biol. Chem.* **178,** *989 (1949)*; *Ibid.* **184,** *563 (1950)*) have indicated that the mucosal block is not effective in pyridoxine deficiency and T. D. Kinney, D. M. Hegsted, and C. A. Finch (*J. Exp. Med.* **90,** *137 (1949)*) showed that it could be overcome by feeding large amounts of iron, most effectively upon a low phosphate diet. The present data suggest that it is also relatively ineffective during protein deficiency.

Since definite changes were observed at the three lower levels of iron intake, 70, 35, and 0 micrograms per day, these represent varying degrees of iron deficiency. On the adequate protein intake the red cell count and the mean corpuscular hemoglobin remained relatively normal while all of the other blood indices fell progressively with the iron intake as did the total body hemoglobin and plasma iron. On the other hand, the nonhemin iron of the liver, spleen and carcass did not decrease appreciably below the level obtained at 70 micrograms of iron per day. The liver then contained 0.91 micrograms per 100 g. of fresh tissue. This is believed to represent no storage iron.

In the protein-deficient groups compared to their own controls that received 280 or 560 micrograms of iron per day, and were already slightly anemic, the changes were similar but somewhat less percentagewise. In comparing the high and low protein groups the author concludes that the limiting effect of protein upon hemoglobin formation is not greater when the iron supply is short, nor does a surplus of storage iron stimulate hemoglobin formation in protein deficiency. Thus the effects of an iron deficiency are simply superimposed upon those of the protein deficiency.

In all of the studies the author noted a fall in adrenal weight which more or less paralleled the fall in total body hemoglobin. Three groups of animals fed a 3.5 per cent protein diet supplemented with 1 mg. of iron daily were used to study the effects of adrenal activity. One group received cortisone, 1.5 mg. per 100 g. body weight daily, and another 3 mg. of ACTH preparation per 100 g. body weight daily. These were compared to the unsupplemented group. The essential finding was that although the injected groups lost considerably more weight than the controls, their total body hemoglobin was significantly higher. The author suggests, therefore, that the loss in total hemoglobin per unit carcass protein which occurs in protein deficiency is secondary to the hormonal adaptation. He notes the lowering of plasma iron following ACTH treatment observed by G. E. Cartwright *et al.* (*J. Clin. Invest.* **30,** *161 (1951)*), although in these experiments both types of treatment resulted in highly significant increases in plasma iron.

Studies upon protein deficiency during pregnancy and lactation in rats, as well as considerable data on the nonhemin iron of human tissues taken at biopsy and necropsy, are also presented, although space does not permit a discussion. The author concludes

that in idiopathic hypochromic anemia a complete lack of storage iron can be demonstrated by analysis of bone marrow obtained by sternal puncture. If any criticism is due of these exceptionally complete studies, it would probably be that the time factor was not studied and comparative groups were left on experiment for different periods of time. Whether this was of any importance in the results obtained remains unknown.

BENTONITE AND LIVER TUMORS IN MICE

The induction of a complex nutritional deficiency in mice by the addition of the clay bentonite to a semipurified ration has been demonstrated by J. W. Wilson (*Ann. N. Y. Acad. Sci.* **57,** *678 (1954)*). The deficiency was in part due to the lack of choline, this substance being bound to and excreted with the clay. Continued maintenance of the mice in the choline-deficient state led to the development of hepatomas in a manner similar to that reported for rats maintained on low protein, low choline rations.

The basal diet consisted of 20 per cent casein, 40 per cent starch, 24 per cent fat, 6 per cent salts, 8 per cent yeast and 2 per cent cod liver oil. Feeding Ruffex (essentially a nondigestible carbohydrate) with the basal diet in 50:50 or 60:40 ratio caused a moderate depression of growth. A 70:30 ratio was fatal, presumably because the animals could not consume enough of this bulky mixture to obtain an adequate caloric intake. The addition of bulk in the form of barium sulfate gave results essentially identical with those of the Ruffex experiments. On the other hand, a mixture of bentonite and basal diet in 50:50 ratio caused a severe depression in the growth rate—a gain of about 5 g. in sixty days on the experimental ration. Histologic examination showed a gradually developing deposition of excess stainable liver fat beginning after about three weeks of feeding the bentonite-containing ration. After several months extensive fatty infiltration had occurred and was accompanied by fibrosis. Proliferation of the remaining liver cells was not pronounced, only occasional mitotic activity being noted in the histologic preparations. Thus the "hob-nail" livers characteristic of choline deficiency were not found. Hepatomas did occur in 11 out of 12 animals maintained on the bentonite ration over two hundred days. This finding suggests that nodular cirrhosis ("hob-nail" cirrhosis) is not a necessary precursor of liver tumors.

Growth on the bentonite-containing diet was considerably improved by the addition of 0.15 per cent choline to the ration. Even with this supplement, however, growth was still far inferior to that observed with the basal ration without bentonite. The author suggests that bentonite, a cation exchange silicate, may combine with choline and render it unavailable to the animal. The fact that choline supplementation did not restore the growth rate to normal may indicate that the clay removed other nutrients such as thiamine and pyridoxine. The added choline was, of course, also in contact with the bentonite and may in part have been rendered unavailable. It would be of interest to note the effect of choline if administered in some way other than by addition to the ration. The choline supplement did effectively prevent the occurrence of fatty livers. Whether the choline supplement also prevented tumor formation was not stated.

Doubling the casein content of the bentonite-containing ratio was as effective as choline supplementation. Growth was stimulated to about the same degree, and fatty infiltration of the liver did not occur. On the other hand, fortifying the diet with methionine at levels of 0.5 and 1.0 per cent was not effective, or was actually injurious. The

mice receiving methionine survived less than ten weeks unless maintained at a slightly elevated temperature (31° C.). This finding is surprising since added methionine might be expected to permit the animal to synthesize choline. Wilson suggests the possibility that basic amino acids may be removed by bentonite and that methionine supplementation may have precipitated an amino acid imbalance. This suggestion seems reasonable since doubling the casein content of the ration was somewhat effective. It should not be difficult to subject the idea to critical experimentation.

It would be of considerable interest to know the nature of the other factors involved in the multiple deficiency caused by bentonite incorporated in the experimental ration. It might also be feasible to speed the induction of certain vitamin deficiencies by removing remaining traces of such vitamins from the alimentary tract or by disposing of vitamins newly synthesized by intestinal microorganisms by means of this substance.

SKIN CHANGES IN NUTRITIONAL DEFICIENCIES

The influence of vitamin A deficiency on epithelial tissue of the rat was reported in detail by S. B. Wolbach and P. R. Howe (*J. Exp. Med.* **42,** *753* (*1925*)). The single-layered epithelium of mucous membranes is replaced by stratified keratinizing epithelium. The skin lesions of vitamin B_6 deficiency were described by J. Goldberger and R. D. Lillie (*Pub. Health Reports* **41,** *1025* (*1926*)). The condition, which at that time was named "rat pellagra," is characterized by a scaly dermatitis most pronounced on the paws, tail and nose. The gross skin lesions of essential fatty acid deficiency in the rat are similar to those resulting from vitamin B_6 deficiency and were described by G. O. Burr and M. M. Burr (*J. Biol. Chem.* **82,** *345* (*1929*)).

There has been considerable effort by dermatologists to relate human skin disorders to malnutrition. This is particularly true of the condition follicular hyperkeratosis, , also called phrynoderma or keratosis pilaris. The condition is characterized by hyperkeratinization of the lining epithelium of the pilosebaceous and sweat glands. In severe cases the skin has a dry scaly appearance and is frequently referred to as toadskin. This disease was attributed to vitamin A deficiency by C. N. Frazier and C.-K. Hu (*Arch. Int. Med.* **48,** *507* (*1931*)). There has been reason to question this conclusion from the standpoint of both histology and nutrition (*Nutrition Reviews* **3,** *170* (*1945*)). Most of the observations of the dermatitis in rats deficient in vitamin A, vitamin B_6 and essential fatty acids have been gross; hence little information is available as to the histologic similarity of the skin in these conditions to that found in human follicular hyperkeratosis. Such information would be of considerable value as a clue to the etiology of the human disease. V. Ramalingaswami and H. M. Sinclair (*Brit. J. Dermat.* **65,** *1* (*1953*)) have reported such a study in which careful histologic examination has been made of the skin of rats deficient in these nutrients.

For the vitamin A experiments rats were given a purified diet containing rice starch, casein, vegetable oil, and salts. The water-soluble vitamins known to be required by the rat were given separately by mouth in some experiments and mixed in the diet in others. L-tocopherol and vitamin D were given by mouth once weekly. In the first experiment acute vitamin A deficiency was induced by feeding the vitamin A-deficient diet for ten weeks to weanling rats. In a second experiment a more chronic deficiency was induced by giving each rat 250 I.U. of vitamin A after ten weeks of feeding and again after twenty weeks. The animals were then killed after a total of twenty-four weeks

on the diet. Control animals for both experiments were given 1000 I.U. of vitamin A each week and were pair-fed with the deficient animals. The deficient animals exhibited the usual signs including growth failure and eye changes. At the termination of the experiment skin samples were taken from the anterior abdominal wall and from the fore and hind limbs for histologic study.

The skin of the deficient animals was abnormal and presented a similar picture in both acutely and chronically vitamin A-deficient rats. The skin was reduced in thickness with atrophy of the basal and Malpighian layers of the epidermis. The stratum granulosum completely disappeared in some places. There was almost complete loss of subcutaneous fat depots. The upper third of the hair follicles were dilated and contained several loosely arranged layers of keratin. There was a reduction in the number of sebaceous glands and those remaining were in various stages of disintegration. These changes are quite different from those reported as accompanying follicular hyperkeratosis in human subjects. In the latter condition the chief characteristics are plugging of the opening of the hair follicles with dense masses of keratin and generalized hyperkeratosis of the epidermis.

In the study of essential fatty acid deficiency weanling albino rats were given a diet consisting of sucrose, casein, salts and the usual vitamins. Crystalline vitamin A acetate and alpha-tocopherol acetate were dissolved in absolute alcohol and administered to the animals. The control animals received this diet modified by the addition of 10 per cent of arachis oil at the expense of sucrose. These diets were fed for twenty-four to thirty weeks, after which time the skin sections were taken for histologic study. The same areas of skin were examined as were used in the vitamin A experiments. The skin changes resulting from fatty acid deficiency were markedly different from those observed in vitamin A deficiency. Sections of skin from the animals deficient in essential fatty acids exhibited marked hyperplasia of the surface epithelium and a well defined layer of fat cells. The keratin layers were closely packed and the openings of the hair follicles were plugged with keratin. The histologic picture of the skin from rats deficient in essential fatty acids resembled strikingly the picture seen in follicular hyperkeratosis in man.

Skin from rats deficient in vitamin B_6 was also examined. The changes resulting from the deficiency consisted of atrophy of the epidermis, loose hyperkeratosis and loss of subcutaneous fat. These changes resemble those observed in vitamin A deficiency and thus differ from the histologic pattern observed in follicular hyperkeratosis.

These experiments show that the histologic picture of follicular hyperkeratosis in man has its counterpart in essential fatty acid deficiency in the rat. In contrast, the skin changes in rats deficient in vitamins A or B_6 differ markedly from those observed in the human disease. It is tempting to speculate that follicular hyperkeratosis is the result of essential fatty acid deficiency. This can be proved only by careful nutritional study and such a study seems to be strongly indicated.

THE ENZYMATIC SYNTHESIS OF COENZYME A

Research in the field of vitamins usually follows a pattern in which various stages of development are apparent. The first stage is usually the demonstration of a nutritional requirement for an unidentified factor by the animal or plant under investigation. This may be followed by the isolation of the factor, elucidation of its chemical nature,

and its chemical synthesis. The last stage consists of assigning a metabolic function to the vitamin. All vitamins the functions of which are established have been shown to be constituents of enzyme systems.

The history of pantothenic acid is an excellent demonstration of this pattern. The vitamin was shown to be a growth factor for yeast and several animal species. Its chemical nature and synthesis were reported later (*see Nutrition Reviews* **12,** *65 (1954)*). The work of Fritz Lipmann and his associates demonstrated that pantothenic acid is a component of coenzyme A. This coenzyme functions in a large number of reactions involving two-carbon compounds and is necessary for reactions which liberate energy and also for many synthetic reactions such as fatty acid synthesis and steroid synthesis. The available data indicate that virtually all the pantothenic acid of tissues occurs in coenzyme A.

The coenzyme A molecule contains pantothenic acid, beta-mercaptoethylamine, adenine, ribose, and phosphoric acid. The coenzyme is readily synthesized by animal tissues provided pantothenic acid is supplied in the diet. The mechanism of this biosynthesis is of interest and has been studied in detail by L. Levintow and G. D. Novelli (*J. Biol. Chem.* **207,** *761 (1954)*) and M. B. Hoagland and Novelli (*Ibid.* **207,** *767 (1954)*).

These workers used an *in vitro* approach in their experiments. Pigeon liver, after various stages of fractionation, was used as the enzyme source. The reactants were incubated with liver fractions and the various intermediates in coenzyme A synthesis were determined by appropriate microbiologic or enzymatic procedures.

The first step in the biologic synthesis of coenzyme A appears to be the formation of pantothenylcysteine. This compound is formed by peptide linkage between the carboxyl group of pantothenic acid and the amino group of cysteine. It was suggested as an intermediate in the synthesis of the *Lactobacillus bulgaricus* factor by G. M. Brown and E. E. Snell (*J. Am. Chem. Soc.* **75,** *2782 (1953)*). Pantothenylcysteine is then decarboxylated to give pantetheine, the *L. bulgaricus* factor (*Nutrition Reviews* **9,** *239 (1951)*). The formation of pantetheine by the liver enzyme used by Hoagland and Novelli (*loc. cit.*) required the presence of pantothenic acid, cysteine, and adenosine triphosphate (ATP).

Pantetheine is next converted to 4′-phosphopantetheine by a liver enzyme which was designated pantetheine kinase. The phosphate group, which becomes attached to the terminal hydroxyl group of the pantothenic acid moiety of pantetheine, is supplied by ATP. Pantetheine was found to be converted to coenzyme A by the pigeon liver enzymes provided ATP, cysteine, and Mg^{++} were added to the reaction mixture. Dephospho-coenzyme A was found to be an intermediate in this conversion.

The sequence of reactions in the biologic synthesis of coenzyme A then appears to be:

(1) Pantothenic acid + cysteine + ATP → pantothenylcysteine
(2) Pantothenylcysteine → pantetheine
(3) Pantetheine + ATP → 4′-phosphopantetheine
(4) 4′-phosphopantetheine + ATP → dephospho-coenzyme A
(5) Dephospho-coenzyme A + ATP → coenzyme A

These experiments present a clear-cut picture of the biosynthesis of coenzyme A. They may indicate an explanation for some nutritional interrelationships of pantothenic acid. It has been shown that rats and chicks deficient in folacin exhibit reduced levels of liver coenzyme A (J. R. Totter, *J. Cell. Comp. Physiol.* **41,** *Suppl. 1, 241 (1953)*). Since coenzyme A contains adenine, and a large body of data indicates that folacin or its derivatives are concerned in purine synthesis, the synthesis of the adenine moiety of coenzyme A may be limiting in folacin-deficient ani-

mals. Several reports have shown that pantothenic acid and methionine are interrelated in nutrition. The beta-mercaptoethylamine portion of coenzyme A would normally be derived from dietary methionine or cystine. If the diet contained inadequate quantities of these amino acids the synthesis of coenzyme A could be impaired.

MALFORMATIONS INDUCED BY VITAMIN A DEFICIENCY

A broad array of congenital malformations in the offspring of vitamin A-deficient rats has been described by Warkany and his associates (J. Warkany, C. B. Roth, and J. G. Wilson, *Pediatrics* **1,** *462 (1948)*; Warkany and E. Schraffenberger, *Arch. Ophth.* **35,** *150 (1946)*). Approximately 75 per cent of the offspring exhibited an anomaly in one or more organs. In decreasing order of frequency the organs involved were the eye, the genitourinary tract, the diaphragm, major arteries derived from the embryonic aortic-arch system, and the lungs. Other anomalies occurred, but they were too infrequent to be considered a part of the syndrome. All of the organs mentioned above showed several kinds of malformations.

J. G. Wilson, C. B. Roth, and J. Warkany (*Am. J. Anat.* **92,** *189 (1953)*) have studied the effect of vitamin A administration on different days of gestation. Weanling female rats were fed a vitamin A-deficient diet. Every tenth day 25 micrograms of carotene were given to insure growth but to prevent any appreciable storage of vitamin A. When regular estrous cycles were established the animals were transferred to a purified diet completely free of vitamin A and allowed to mate. Pregnancy was dated from the day that sperms were found in the vaginal smears. One group of females was given no vitamin A and other groups were given one dose of 16,000 I.U. of vitamin A on the tenth, eleventh, twelfth, thirteenth, fourteenth, or fifteenth day of pregnancy. The animals were killed upon the twentieth to twenty-second day of pregnancy and all fetuses were fixed in formalin. One fetus from each litter was prepared for histologic study. Of 439 females which were inseminated only 261 carried their conceptus (fetuses) to a time at which they could be examined. The remaining 178 females either did not become pregnant or resorbed the embryos. Only those animals which went to the twentieth to twenty-second day of pregnancy without overt signs of embryonic death (loss of weight, excessive blood in the vagina) were included in the study. The size of the groups on which the study is based varied from 21 to 50 females and, as indicated, one rat from each of these litters was examined.

The primordium of the eye is first recognized on the eleventh day as a shallow evagination of the forebrain. By the fourteenth day the cup is deeply indented by the choroid fissure and the fissure is closed behind the hyloid artery on the fifteenth day. Not until the eighteenth day do the lids, ciliary body and retina show appreciable differentiation. The principal ocular defects observed in the vitamin A-deficient animals include postlenticular fibroplasia, incomplete closure of the choroid fissure and eversion of the nervous layer of the retina. All of these defects were prevented by administering vitamin A on the tenth, eleventh, and twelfth days of pregnancy. Only 2 animals dosed upon the thirteenth day showed these defects and the incidence was reduced, as compared to untreated females, by dosage upon the fourteenth and fifteenth days. It should be noted that there is some doubt as to whether controls of this type are completely adequate and comparative, *i.e.*, animals which received no vitamin A. Undoubtedly treatment with vitamin A allows some females to carry their young to term when they otherwise might be resorbed. This seemed particularly true of the aortic arch

anomalies, to be discussed in the following paragraph, since the animals which received vitamin A had a higher incidence of anomalies than the untreated controls. A total of 10 animals treated upon the tenth day of pregnancy had ocular anomalies classified as "other than those typical" which included open lids, no lids, cataracts, etc. One instance of anophthalmia (absence of eyes) and one of microphthalmia (abnormally small eyeball) were seen in this group, defects not previously observed in vitamin A deficiency. Thus such defects are probably due to early or severe deficiency in which the fetus fails to survive unless therapy is given early.

All anomalies of the aortic arches were prevented by vitamin A administration upon the tenth or eleventh days, but there was apparently little effect after that time. The over-all incidence was 15 per cent of the animals dosed upon the twelfth day, 14 per cent of the animals dosed on the thirteenth day, and 17 per cent on the fourteenth and fifteenth days. As indicated above, the animals without therapy showed an over-all incidence of anomalies of the aortic arch of only 9 per cent. As in the eye anomalies the treated rats showed a high incidence of unusual anomalies, indicating that they had been salvaged by the therapy. It seems apparent that the abnormal pattern of the aortic arches had been established by the twelfth day, although in the normal embryo it is not until the fifteenth day that the third, fourth, and sixth pairs which figure prominently in the definitive pattern begin to assume some of their characteristic relationships.

Defects of the heart were apparently reduced by dosage upon the tenth, eleventh, and twelfth days (5 to 8 per cent anomalies), but not appreciably by dosage after this date (13 to 25 per cent defects). This is perhaps consistent with the fact that the first cardiac primordium is recognized upon the tenth day and on the thirteenth day a primitive four-chambered configuration is attained. Again the high incidence of as many as 25 per cent of the embryos with cardiac defects compared to only 4 per cent in the untreated controls indicates the necessity of a reasonably functional heart if the fetus is to survive to term. As many as 8 per cent of the animals treated upon the twelfth day had anomalies of a type which did not occur in the untreated group.

Defects of the lungs did not occur when vitamin A was given before the twelfth day. Similarly, diaphragmatic hernia was prevented by dosage upon the tenth and eleventh days. In the latter defect an incidence of 8 and 16 per cent was observed when the vitamin was given on the twelfth and thirteenth days respectively and 24 per cent when it was given on the fourteenth or fifteenth day. The untreated controls showed an incidence of 31 per cent. Thus it appears that even dosage as late as the fifteenth day was partially effective. It is of interest that in the treated animals most of the hernias occurred upon the left side only, and only animals dosed upon the fourteenth and fifteenth days had an appreciable incidence of hernia on the right side. All of the untreated controls had the hernia on the right side only or on both sides. Thus there seemed to be a definite shift depending upon the length of the deficiency and the authors comment that this cannot be explained by the known events of the embryonic development of the diaphragm.

The metanephroi first appear as buds upon the mesonephric ducts on the thirteenth day. Vitamin administration prior to this time completely prevented renal defects with the exception of hydronephrosis. The latter anomaly was not observed in the untreated animals and showed a curious pattern. There was a 10 per cent incidence in animals treated upon the tenth day, completely absent in animals treated upon the twelfth day, and the incidence rose to 13 per cent in animals treated upon the fifteenth day. This is not considered strictly as a developmental defect of the kidney and is ascribed to some other cause, possibly obstruction of the lower

urinary tract, either anatomic or functional in nature. Over-all, the renal abnormalities showed a much lower incidence in the treated animals, even those treated on the fifteenth day, than the untreated controls. The effect of vitamin administration upon abnormalities of the ureters was similar to the effects observed on the kidneys. Animals dosed prior to the thirteenth day, the day the uretric buds appear, produced no young with defects other than hydroureter (ureter abnormally distended with urine). This defect was not observed in the untreated controls and occurred in highest incidence in animals dosed upon the tenth day. Between the thirteenth and seventeenth days of gestation the terminal orifice of the ureter moves distally. The most common defect noted was ectopic termination of the ureters and dosage upon the fourteenth day markedly decreased the incidence while those animals dosed upon the fifteenth day had a high incidence.

The abnormalities encountered in the genital ducts were completely prevented by dosage prior to the twelfth day of pregnancy. Agenesis or incomplete development of the Mullerian ducts was encountered in all of the other groups (although in considerably lower incidence than in the untreated controls). These findings were unusual since these structures do not begin their development until the fifteenth day and are not complete until the seventeenth day. Thus there was little or no correlation between the preventive action of vitamin A and the time of development of the organs.

It thus appears that the administration of vitamin A at progressively earlier times in deficient females progressively decreases the incidence of abnormalities; that the types of malformation are modified by therapy on particular days of pregnancy; and that the teratogenic effects of vitamin A deficiency are exerted primarily on the process of organogenesis rather than upon the presumptive but undifferentiated tissue. The correlation between organogenesis and effect of therapy is close in some tissues but only moderately so in others. Only in the case of the genital ducts was there indication that the effect of the deficiency might have affected the tissue prior to organogenesis.

It is apparent that other types of teratogenic agents do not act similarly. C. R. Stockard (*Am. J. Anat.* **28,** *115 (1921)*) showed that with sea minnows the effect of low temperature or oxygen supply was most active in producing abnormalities in the blastopore stage, *i.e.*, before organ formation begins. X-irradiation produces numerous abnormalities in rat embryos when applied before the embryonic elements of the organs are recognizable. Thus congenital malformations may originate at three different times at least: before fertilization by germ-cell mutations, prior to organogenesis, or during the period of organogenesis.

To the unprofessional observer of this field it would appear that the conclusion that vitamin A is primarily active during organogenesis should be accepted with some reservations. These data were obtained only upon animals which survived to term and the incidence of unusual abnormalities was high in the animals treated early with vitamin A. Some of the defects were clearly established by the tenth day of pregnancy. As in most research, the excellent work of Warkany and his associates has outlined as many new problems as it has solved.

NOTES

The Chemical Nature of "Active Methionine"

Methionine serves two major functions in animal metabolism. It is a constituent of tissue proteins and it also serves as a methyl donor in transmethylation reactions. H. Borsook and J. W. Dubnoff (*J. Biol. Chem.* **171,** *363 (1947)*) were able to demonstrate that adenosine triphosphate (ATP) was required for methionine to be utilized in the methylation of guanidoacetic acid to creatine. Subsequently it was shown by G. L. Cantoni (*Ibid.* **189,** *745 (1951)*) that methionine reacted with ATP to form an intermediate compound which in turn was the immediate methyl donor for creatine synthesis. This intermediate compound was called "active methionine."

Evidence has now been presented by G. L. Cantoni (*J. Biol. Chem.* **204,** *403 (1953)*) that "active methionine" is methyl (5-desoxyribosyladenine) (2-aminobutyro) thetin. For the sake of brevity the compound is referred to as S-adenosylmethionine and consists of methionine linked through its sulfur to the fifth carbon atom of the ribose of adenosine. The preparation of active methionine used for the chemical characterization studies was obtained enzymatically. A rabbit liver preparation was incubated with L-methionine and ATP. "Active methionine" was isolated from the incubation mixture and was purified by paper chromatography technic. It was found that when the methionine added to the incubation mixture was labeled with S^{35} the "active methionine" which was found contained the S^{35}, thus showing that methionine retained its sulfur while being converted to the active compound. Adenine was identified among the hydrolysis products of the material and the molar ratio of labile methyl, adenine, and pentose was 1:0.78:0.8. Since the ratio should have been 1:1:1 for the proposed structure it appeared that the material was about 80 per cent pure in terms of adenine content. The evidence for the proposed linkage between the methionine and adenosine was chiefly circumstantial. However, when it is considered that this type of linkage exists in 5′-thiomethyladenosine (K. Satoh and K. Makino, *Nature* **167,** *238 (1951)*), and that the S—CH_3 of this compound is derived from methionine (R. L. Smith and F. Schlenk, *Fed. Proc.* **11,** *289 (1952)*), the proposed linkage in S-adenosylmethionine appears quite reasonable.

Effect of Temperature on Tomato Color

It has long been recognized that lycopene development in tomatoes is dependent on temperature. Lycopene does not develop below 50° nor above 86°F., as shown by experiments conducted under constant temperature in the dark, with detached fruit. Lycopene is important because the market demands red tomatoes. Recent experiments by C. B. Sayre, W. B. Robinson, and T. Wishnetsky (*Proc. Am. Soc. Hort. Sci.* **61,** *381 (1953)*) with greenhouse tomato plants exposed to sunlight have used more natural conditions for which temperature is the only variable and fruits are left on the vines. Fruits were carefully identified for comparison regarding age; they were also protected from direct sunlight. Temperature conditions were as follows: low (60°F. day, 45°F. night), medium (75°F. day, 60°F. night), and high (100°F. day, 85°F. night). Comparable detached fruits were also included for comparison. Two varieties were studied, one of them during two seasons.

Surface color, measured by a color-difference meter, was reduced 60 per cent at the high temperature, and the color of the raw juice was reduced ten- to fifteen-fold at the high temperature. Lycopene content was greater by 20 to 40 per cent at the medium temperature than at the low temperature and it was only about one-eighth as great at the high temperature as at the

medium temperature. Carotene content was about the same at all these temperatures. Ascorbic acid contents were greatest at the low temperature. No great differences were observed between the detached and the vine-ripened fruits.

The medium-temperature fruits ripened in nine days to well-colored specimens. Tomatoes grown at the low temperature required twelve to fifteen days to ripen and the fruit usually became soft and moldy. At the high temperature the color was yellow or orange, and the fruits would grade as culls. Even though temperatures exceed 85°F. for part of each day, field-grown tomatoes will develop some lycopene. When the high-temperature plants were moved to the medium-temperature conditions, lycopene developed quickly and better fruits were soon produced.

It is refreshing to see laboratory studies include more natural conditions, as in this work on what is perhaps the most important psychologic factor to the consumer of tomatoes, their color.

Mushrooms Grown by Submerged Culture

The possibility of growing mushroom myceliums in submerged culture, after the methods employed in the antibiotic industry, was investigated by S. S. Block, T. W. Stearns, R. L. Stephens, and R. F. J. McCandless (*J. Agr. Food Chem.* **1,** *890 (1953)*). Large quantities of low-cost potential growth mediums are available in the form of citrus press water, waste from the production of citrus juices. This fungus will grow well on inorganic mediums supplemented with glucose alone. Buffer problems are simple in synthetic mediums, since the mycelium soon brings the pH of the medium, whether acid or basic, to 5.5. However, on orange juice medium the pH rises from acid values to pH 8.0. The product, centrifuged from the medium, is a pasty, cream-colored substance resembling compressed yeast. Stored juices (unless frozen) develop toxic factors that prevent growth, while fresh juice compares favorably with well-known growth mediums such as malt extract and corn steep liquor.

The protein content of mushrooms is high, around 40 per cent on a dry basis. The amino acid constituents of both the mycelium and the fruiting bodies were determined by paper chromatography. Qualitatively these materials compare favorably with other common proteinaceous food materials. Compared to the edible portion of the whole wheat kernel, the mushroom mycelium is rich in the B-vitamins. These constituents might well be increased in quantity by further study of the effects of variation and culture conditions on growth and composition.

When grown on synthetic mediums the myceliums have little flavor. On orange juice the flavor is bitter, a taste which can be removed by washing with water. Heating to 75° C. causes autolysis and improvement of the flavor, which does not resemble that of fresh-cooked mushrooms. Soups made from this mycelium, however, did resemble canned mushroom soups in flavor. Production of better flavor remains the primary problem before commercial production in submerged culture becomes practical.

Recent Nutrition Books

Control of Tooth Decay. Food and Nutrition Board, National Research Council, Washington, D. C., 1953. Pp. 18. Price: $0.20 each; 6–50 copies, $0.10 each; over 50 copies, $0.05 each. Copies obtainable from the Division of Biology and Agriculture, National Research Council, 2101 Constitution Ave., N.W., Washington, D. C.

Recommended Dietary Allowances, Revised 1953. National Academy of Sciences-National Research Council Publication No. 302, Washington, D. C., 1953. Pp. 36. Price: $0.50.